

US Serial No. 09/060,872
Page 2

LISTING OF CLAIMS:

The following is a marked-up version of the Claims, provided pursuant to 37 C.F.R. §1.121 (c)(1)(ii) with instructions and markings showing changes made herein to the previous version of the Claims on record. Underlining denotes added text while strikeout denotes deleted text.

1. (Cancelled)
2. (Cancelled)
3. (Cancelled)
4. (Cancelled)
5. (Cancelled)
6. (Cancelled)
7. (Cancelled)
8. (Cancelled)
9. (Cancelled)
10. (Cancelled)
11. (Cancelled)
12. (Cancelled)
13. (Cancelled)
14. (Cancelled)

US Serial No. 09/060,872
Page 3

15. (Cancelled)

16. (Cancelled)

17. (Currently Amended) A method for determining a T-cell epitope of a peptide, comprising the steps of:

- (a) obtaining from a single human blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells;
- (b) differentiating said dendritic cells in the presence of cytokines;
- (c) combining said solution of differentiated dendritic cells and said naïve CD4+ and/or CD8+ T-cells with the peptide, said peptide comprising said T-cell epitope; and
- (d) measuring proliferation of said T-cells in said step (c).

18. (Currently Amended) A method of reducing the allergenicity of a protein comprising the steps of:

- (a) identifying a T-cell epitope in said protein by
 - (i) contacting an adherent monocyte-derived dendritic cell that has been differentiated by exposing said dendritic cell to cytokines, with a peptide comprising said T-cell epitope; and
 - (ii) contacting said dendritic cell and peptide with a naïve T-cell, wherein said naïve T-cell has been obtained from the same source as said adherent monocyte-derived dendritic cell, and whereby said T-cell proliferates in response to said peptide; and
- (b) modifying said protein to neutralize said T-cell epitope such that the modified protein induces less than or substantially equal the baseline proliferation of said naïve T-cells.

19. (Previously Added) The method according to claim 18, wherein the protein is a protease.

US Serial No. 09/060,872
Page 4

20. (Previously Amended) A method for reducing the allergenicity of a microbial subtilisin comprising the steps of:

- (a) determining a T-cell epitope of said subtilisin comprising (i) obtaining from a single human blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells; (ii) promoting differentiation in said solution of dendritic cells; combining said solution of differentiated dendritic cells and said naïve CD4+ and/or CD8+ T-cells with peptide fragments of said subtilisin; and (iv) measuring proliferation of said T-cells in said step (iii); and
- (b) modifying the peptide which includes the T-cell epitope to neutralize said epitope.

21. (Previously Added) The method according to claim 20, wherein the microbial subtilisin is derived from a *Bacillus*.

22. (Previously Added) The method according to claim 21, wherein the *Bacillus* is selected from the group consisting of *B. lentus*, *B. subtilisin*, *B. amyloliquefaciens* and *B. licheniformis*.

23. (Previously Amended) The method according to claim 20, wherein said epitope of said microbial subtilisin is modified by: (a) substituting the amino acid sequence of the epitope with an analogous sequence from a human homolog of said microbial subtilisin; (b) substituting the amino acid sequence of the epitope with an analogous sequence from a non-human homolog of said microbial subtilisin; or (c) substituting the amino acid sequence of the epitope with a sequence which substantially mimics the major tertiary structure attributes of the epitope.

24. (Cancelled)

25. (Cancelled)

26. (Cancelled)

US Serial No. 09/060,872
Page 5

27. (Cancelled)

28. (Cancelled)

29. (Previously Added) The method according to claim 18, wherein said T-cell epitope is modified by a substitution selected from the group consisting of:

(a) substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a human homolog to the protein of interest;

(b) substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a non-human homolog to the protein of interest; or

(c) substituting the amino acid sequence of said T-cell epitope with a sequence which substantially mimics the major tertiary structure attributes of the epitope.

30. (Previously Added) The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of the T-cell epitope with an analogous sequence from a human homolog to the protein of interest.

31. (Previously Added) The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a non-human homolog to the protein of interest.

32. (Previously Added) The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of the epitope with a sequence which substantially mimics the major tertiary structure attributes of said T-cell epitope.

33. (New) A method for determining a T-cell epitope of a protein, comprising the steps of:

(a) obtaining a protein and preparing peptide fragments of said protein;

(b) obtaining from a single human blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells;

US Serial No. 09/060,872
Page 6

(c) promoting differentiation of said dendritic cells by exposing said dendritic cells to granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4);

(d) combining said solution of differentiated dendritic cells and said naïve CD4+ and/or CD8+ T-cells with said peptide fragments, wherein said peptide fragments comprise said T-cell epitope; and

(e) measuring proliferation of said T-cells in said step (d).

34. (New) The method of Claim 33, wherein said promoting differentiation of said dendritic cells in step (c) further comprises exposing said dendritic cells to tumor necrosis factor alpha (TNF- α).

35. (New) The method of Claim 33, wherein said promoting differentiation of said dendritic cells in step (c) further comprises exposing said dendritic cells to interleukin-1 alpha (IL-1 α).

36. (New) A method of reducing the allergenicity of a protein comprising the steps of:

(a) identifying a T-cell epitope in said protein by

(i) contacting an adherent monocyte-derived dendritic cell that has been differentiated in the presence granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4), with a peptide comprising said T-cell epitope; and

(ii) contacting said differentiated dendritic cell and peptide with a naïve T-cell, wherein said naïve T-cell has been obtained from the same source as said adherent monocyte-derived dendritic cell, and whereby said T-cell proliferates in response to said peptide; and

(b) modifying said protein to neutralize said T-cell epitope such that the modified protein induces less than or substantially equal the baseline proliferation of said naïve T-cells.

37. (New) The method of Claim 36, wherein said contacting said monocyte-derived dendritic cell in step (a)(i) further comprises exposing said dendritic cell to tumor necrosis factor alpha (TNF- α).

US Serial No. 09/060,872
Page 7

38. (New) The method of Claim 36, wherein said contacting said monocyte-derived dendritic cell in step (a)(i) further comprises exposing said dendritic cells to interleukin-1 alpha (IL-1 α).

39. (Previously Added) The method of Claim 36, wherein said protein is a protease.